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The Impact of Lead-contaminated Soil on *Eruca sativa* Plants

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Abstract

Heavy metal contamination is a major environmental concern due to its detrimental effects on soil and water quality as well as human health. This study aimed to evaluate the effects of lead (Pb^{2+}) contamination on seed germination, morphological growth, and physiological characteristics of *Eruca sativa* and to assess its tolerance to Pb^{2+} stress. The experiment was conducted in a private field at the Biology Department, Koya University, Erbil, Kurdistan Region of Iraq, beginning on October 26, 2025. Three Pb^{2+} concentrations (0, 25, and 50 $mgkg^{-1}$), applied as $PbCl_2$, were used to treat the soil. The results showed that the soil pollutants with Pb^{2+} significantly reduced seed germination and adversely affected several vegetative growth parameters, including plant height, stem diameter, leaf area and root length compared with the control treatment. In contrast, Pb^{2+} application had no significant effect on the number of leaves, fresh mass of shoot, fresh and dry mass of root, as well as the contents of chlorophyll a, chlorophyll b, and total carotenoids. Lead exposure also significantly altered specific stomatal characteristics, including the stomatal density and width of the abaxial surface and the stomatal width of the adaxial surface. Furthermore, increasing the Pb^{2+} concentration to 50 $mgkg^{-1}$ reduced the shoot metal tolerance index, indicating decreased tolerance to Pb^{2+} stress. These findings demonstrate that Pb^{2+} contamination negatively affects the germination and growth of *E. sativa*, while some physiological traits remain relatively unaffected, suggesting differential sensitivity among plant characteristics under Pb^{2+} stress.

Keywords: phytoremediation, brassicaceae, heavy metal and lead

1. Introduction

Eruca sativa, often referred to as rocket, arugula, or garden rocket, is a green leafy plant that is part of the Brassicaceae (mustard) family. It is recognized not only as a consumable salad ingredient but also for its capacity in phytoremediation, thanks to its tolerance and accumulation of specific heavy metals like lead, cadmium, and zinc (Zheng et al., 2025).

Phytoremediation describes the removal of pollutants from the environment (soil, water, and air) through the action of plants. Plants establish a symbiotic relationship with microorganisms that aids in soil remediation, especially concerning heavy metals and organic contaminants. Phytoremediation is often regarded as an eco-friendly technology due to its remarkable capacity to remove heavy metals while producing minimal secondary waste in the environment. On the other hand, phytoremediation is widely favored by the public because of its simple implementation, affordability, and eco-friendly characteristics. Phytoremediation encompasses several processes, including Phytoextraction, Phytostabilization, Phytodegradation, Rhizodegradation, Phytovolatilization, and Rhizofiltration (Kafle et al., 2022 & Alsafran et al., 2022). Heavy metals refer to chemical elements with a density exceeding 5 g/cm3. Heavy metals are prevalent in the environment due to both natural and human activities, leading to human exposure via multiple pathways. Irrigating with wastewater, disposing of solid waste, applying sludge, vehicle emissions, and industrial processes are primary contributors to soil pollution with heavy metals, leading to a heightened absorption of metals by food crops cultivated in these contaminated soils. Overall, wastewater holds significant quantities of valuable nutrients and harmful heavy metals, presenting both opportunities and challenges for agricultural output (Briffa et al., 2020 & Ondrasek et al., 2025).

Lead is a dense metal that does not decompose or deteriorate in nature, making it possible for it to stay in the soil for years or even hundreds of years, threatening plants, wildlife, and people. Lead is a toxic heavy metal that is non-essential, and its effects on biological systems are consistently harmful. Soil lead contamination indicates the existence of harmful amounts of lead within the soil (Mustapha et al., 2026).

This study aims to explore the impacts of Pb^{2+} as a soil pollutant on the seed germination, along with various morphological and physiological characteristics of *Eruca sativa*.

2. Materials and Methods

2.1 Plant Resources and Experimental Design

A pot experiment was conducted in a private field of the Biology Department at Koya University, in the Iraqi Kurdistan Region, situated at 44°38' E, 36°4 N, and an altitude of 570 m above sea level. A complete randomized design (CRD) with three replications was employed to investigate the effects of lead (Pb^{2+}) levels of 0, 25, and 50 $mgkg^{-1}$ soil on *Eruca sativa* seeds. The healthy seeds were sourced from the Koya city market, and were sown on October 26th, 2025 at a depth of 2.5 cm in black polyethylene bags (10x10x30 cm) filled with 3 kg of loamy soil, which was well combined with designated concentrations of $PbCl_2$ labeled as Pb0, Pb25, and Pb50 for the respective lead salt. The bags were adequately irrigated with tap water to ensure potentially uniform soil moisture levels. To prevent loss of nutrients and trace elements out of the experimental units, unperforated polythene sheets

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were placed under each one (figure 1). Newly planted plants were covered with polyethylene (plastic) bags to protect them from winter rainfall.

Fig 1. *E. sativa* plants four months aged

2.2 Meteorological Data

Table (2.1) presents the maximum and minimum temperatures, relative humidity, and rainfall amounts in the open field during the planting season as recorded by the Agro-Meteorological Station in Koya.

Table 2.1. Maximum and minimum temperature, the relative humidity and the amount of rain fall during the growing season

Month (2025-2026)	**Air Temp. C°**		**Relative Humidity%**		**Rain fall (mm)**
	Maximum	**Minimum**	**Maximum**	**Minimum**	
October	28	18	45	35	5-10
November	22	12	65	50	120-150
December	18	8	68	55	90-110
January	15	6	78	65	95-115
February	16	7	68	55	140-160

2.3 Studied Characteristics

2.3.1 Germination Percentage (%)

The germination was calculated depending on the formula (Ullah et al., 2024):

$$G (\%) = L/S \times 100$$

G (%) = Germination percentage
L = Overall count of seedlings that have emerged
S = Quantity of seeds sown

2.3.2 Morphological Traits

The traits were measured after four months of seed germination.

Various growth traits as height of plant (cm), diameter of stem (cm), count of leaves per plant, length of primary root (cm), area of leaf (cm^2), fresh mass of shoot (g), dry mass of shoot (g), fresh mass of root (g), dry mass of root (g), (Khudhur & Qader, 2026 & Qi et al., 2019).

2.3.3 Physiological Traits

Evaluation of the photosynthetic pigment levels: The amount of chlorophyll a, b and total carotenoids were estimated using method 80% acetone as mentioned by (Lichtenthaler & Wellburn, 1983). Mixture ratio was 50 ml: 1 g sample, where 0.4g of fresh leaves were mixed with 20 ml 80% acetone then grinded by mortar and pestle and filtered by filter paper. The extraction placed in a 25 ml glass vial (closed dark bottle), to prevent evaporation and avoid photo-oxidation of pigments, and then read the results by spectrophotometer at 663, 646 and 470 nm wave lengths. Chlorophyll a, chlorophyll b and total carotenoids were calculated as follows:

$$\text{Chl a (mg/L)} = (12.21 \times A663) - (2.81 \times A646)$$

$$\text{Chl b (mg/L)} = (20.13 \times A646) - (5.03 \times A663)$$

$$\text{Total carotenoids (TC) (mg/L)} = (1000 \times A470 - 3.27 \times \text{Chl a} - 104 \times \text{Chl b}) / 229$$

To change the concentration from mg/L to mg/g fresh weight, multiply each value by (extraction volume / sample weight) ×1000.

2.3.3.1 Stomatal Features

3^{rd} - 4^{th} full matured healthy leaves were taken and kept in polythene bags. The lasting impressions method was used to leaf epidermal peel slides. In this method, at least one square centimeter on leaf surface was painted by a thick patch of clear nail polish. Allowing the nail polish to dry completely then taped a piece of clear cellophane tape to the dried nail polish patch by carton sealing tape. Then, peeling out the nail polish patch by pulling a corner of the tape and the finger nail polish along with the leaf peel. This leaf impression which was taped on slides and labeled as adaxial and abaxial surfaces then the samples were observed under a light microscope (DM 300, Leica Microsystems, China) with camera attached using Analysis Image with Analysis Software (Khasanah & Na'ima., 2022).

- **Number of stomata:** Slides of leaf impression was examined under 40x by light microscope. Numbers of appeared stomata on lens field were counted for each adaxial and abaxial leave surface.
- **Length and width of stomata:** Length and width of stomata guard cells of adaxial and abaxial leaf surfaces were measured in micron with scaled ocular lens.

2.3.3.2 Metal Tolerance Index (MTI)

The metal tolerance index (MTI) is an essential measure in phytoremediation research that assesses a plant's capacity to endure metal toxicity. The method of the metal tolerance index (MTI) used in this study was borrowed from (Herlina et al., 2020). Equation for metal tolerance index (MTI).

$$\textbf{MT1 (\%)} = [(\text{biomass of treated plant}) / (\text{biomass of control})] \times 100$$

2.4 Statistical Evaluation

The experiment was carried out following a completely randomized design (CRD). Where the concentrations of the element Pb^{2+} was regarded as the factor. Six bags served as a single experimental unit, with three repetitions for each treatment. The means of treatment were compared through the analysis of variance (ANOVA) utilizing the SAS statistical software version 9.1. Duncan's multiple range ($\alpha \leq 0.05$) was utilized for comparing treatment means (Al-Mohammadi & Al-Mohammadi, 2002).

3. Results and Discussions

3.1 Impact of Lead (Pb^{2+}) on Seed Germination and Vegetative Growth Traits of E. Sativa Plants

The results of tables 1 and 2 show that Pb^{2+} application to the soil was significant effects on the seed germination and morphological characteristics of *E. sativa.* Concerning vegetative growth, Pb^{2+} concentrations significantly reduced plant height, stem diameter, leaf area and root length in the 25 and 50 $mgkg^{-1}$ treatments compared to the control. Also, some traits of the vegetative growths were not significant such as the number of plant leaves, fresh mass of (shoot and root) and dry mass of (shoot and root). The increase in the Pb^{2+} application to the soil was significant effects on the seed germination and morphological characteristics concentrations, accompanied by gradual decreases in plant height, stem diameter, plant leaf area and root length. Application of Pb^{2+} application to the soil was significant effects on the seed germination and morphological characteristics at 25 or 50 $mgkg^{-1}$ reduce germination of seeds with 17.11% an 34.65%, respectively compared with Pb^{2+} application to the soil was significant effects on the seed germination and morphological characteristics untreated plants.

The findings indicate that the plants experiencing heavy metal stress exhibited a distinct reduction in growth characteristics relative to the control, probably because of the high concentrations of heavy metals in the soil, which limited nutrient uptake by the plants, leading to reduced growth. Lead is a harmful heavy metal that is not essential and adversely impacts plant growth and metabolism. Exposure to lead hampers seed germination and lowers root and shoot growth by interfering with cell division and influencing cell wall development (Khan et al., 2022 & Zhi et al., 2015). It disrupts photosynthesis by lowering chlorophyll production and harming chloroplast structure, resulting in less energy generation and inhibited growth. Lead additionally modifies enzyme functions and induces oxidative stress by increasing the production of reactive oxygen species, leading to cellular harm. Moreover, it rivals and hinders the absorption of vital nutrients like calcium, magnesium, iron, and zinc, which further disrupts normal physiological functions. The majority of absorbed lead builds up in roots, restricting the uptake of water and nutrients, which ultimately decreases plant productivity and yield (Gupta et al., 2024).

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Table 1. Effects of lead on seed germination and vegetative growth of *E. sativa*

Treatments	Germination Percentage (%)	Plant height (cm)	Stem diameter(cm)	Plant leaves number	Plant leaf area (cm^2)	Root length(cm)
T0 Pb0	91.33^a	8.07^a	0.37^a	8.90^a	22.50^a	24.67^a
T1 Pb 25	75.67^b	4.77^b	0.20^b	9.43^a	14.73^b	14.43^{ab}
T2 Pb 50	59.67^c	4.87^b	0.37^a	9.43^a	21.93^a	7.57^b

* Means followed by the same letters within columns are no significantly different at $p \le 0.05$ according to the Duncan test, and vice versa.

Table 2. Effects of lead on the fresh and dry mass (shoot and root) of *E. sativa*

Treatments	Fresh mass of shoots (%)	Dry mass of shoots (%)	Fresh mass of roots (%)	Dry mass of roots (%)
T0 Pb 0	6.70^a	5.90^a	1.47^a	1.30^a
T1 Pb 25	4.70^a	3.53^a	1.33^a	1.27^a
T2 Pb 50	8.43^a	4.33^a	2.17^a	1.80^a

* Means followed by the same letters within columns are no significantly different at $p \le 0.05$ according to the Duncan test, and vice versa.

3.2 Effect of Lead on Physiological Properties of E. Sativa Plants

3.2.1 Photosynthetic Pigments

Results in the table 3 show that the effect of Pb^{2+} application was non - significant on some photosynthetic pigments for the *E. sativa,* that including chlorophyll a, chlorophyll b and total carotenoids.

Table 3. Effects of lead on photosynthesis pigments of E. sativa plants

Treatments	Chl_a	Chl_b	TC
	mg /g fresh weight		
T0 Pb 0	0.12^a	0.04^a	0.02^a
T1 Pb 25	0.11^a	0.04^a	0.02^a
T2 Pb 50	0.11^a	0.04^a	0.02^a

* Means followed by the same letters within columns are no significantly different at $p \le 0.05$ according to the Duncan test, and vice versa.

3.2.2 Effect of Lead on Stomata Characteristics

Results in tables 4 show that Pb^{2+} application to the soil was significant effects on the seed germination and morphological characteristics treatments significantly affected certain stomatal characteristics, including the number and width of stomata on the abaxial (lower) surface and the width of stomata on the adaxial (upper) surface of *E. sativa*. With Pb^{2+} applications, certain stomatal traits were diminished. The measurements for the width of stomata on the adaxial and abaxial surfaces (0.45 and 0.85 microns) were reduced upon adding Pb^{2+} treatments at 50 $mgkg^{-1}$ compared to the control treatments. The only exception

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was that the number of stomata on the abaxial surface increased with higher Pb concentrations at 25 $mgkg^{-1}$. The maximum value of 83.0 was observed at 25 $mgkg^{-1}$ in comparison to the control treatment (54.7)

The lead is one of the most common heavy metal contaminants in soils. Plants are exceedingly harmful to it. Pb has no biological purpose in plants, although it can create morphological, physiological, and biochemical problems. Plants can take in lead from the soil via water absorption by their roots or through their leaf stomata. Elements affecting the lead levels in plants consist of the exposure time to lead, the lead concentration in water, the plant's morphology and physiology, alongside the specific plant species (Begum, 2026). Significant amounts of accumulated lead in leaves can impact the stomatal function of plants (Bazvand et al., 2025 & Hamidah et al., 2024).

Table 4. Effects of lead on some stomata characteristics of *E. sativa*

Treatments	**Adaxial stomata**			**Abaxial stomata**		
	Number	**Length**	**Width**	**Number**	**Length**	**Width**
	Micron					
T0 Pb 0	39.00^{a}	1.00^{a}	0.65^{a}	54.67^{b}	1.00^{a}	0.55^{b}
T1 Pb 25	39.00^{a}	1.10^{a}	0.50^{ab}	83.00^{a}	1.00^{a}	0.45^{b}
T2 Pb 50	16.33^{a}	1.15^{a}	0.45^{b}	62.67^{ab}	0.95^{a}	0.85^{a}

* Means followed by the same letters within columns are no significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

3.2.3 Metal Tolerance Index (MTI)

Results in table 5 indicate that the impact of Pb^{2+} application significantly reduced the metal tolerance index (MTI) of shoots in *E. sativa* as Pb^{2+} concentrations increased. The buildup of lead levels in the plant tissues of *E. sativa* decreased at concentrations of 25 and 50 $mgkg^{-1}$ for the shoot MTI, with values of 61.03% and 43.17%, respectively, in comparison to the control treatments. While the application of lead had no significant effect on the root's MTI. The accumulation of Pb^{2+} is greater in roots than in shoots.

The excessive buildup of heavy metals in agricultural soils due to wastewater irrigation can lead to soil contamination and increased heavy metal absorption by crops, consequently impacting food quality and safety. The buildup of heavy metals in soils and vegetation is a growing worry due to the possible risks to human health. The contamination of this food chain represents a significant route for these harmful pollutants to enter the human body. The accumulation of heavy metals in plants varies by species, and the effectiveness of various plants in metal absorption is assessed through either plant uptake or the transfer factors of metals from soil to plant. Phytoremediation is a well-recognized, environmentally friendly, and low-cost approach for decontaminating soils polluted with heavy metals, employing plants to absorb, stabilize, or neutralize pollutants. The effectiveness of this eco-friendly technology depends significantly on choosing plants that have substantial biomass, rapid growth rates, and a strong tolerance to heavy metals, frequently assessed through the Metal Tolerance Index (Liu et al., 2022 & Mahmud et al., 2026 & Chrysargyris et al., 2023 & Kaur et al., 2023).

Table 5. Effects of lead on metal tolerance index (MTI) of shoot and root for *E. sativa*

Treatments	**MTI of Shoot (%)**	**MTI of Root (%)**
T0 Pb 0	63.93^{a}	68.20^{a}
T1 Pb 25	61.03^{a}	56.27^{a}
T2 Pb 50	43.17^{b}	52.83a

* Means followed by the same letters within columns are no significantly different at $p \leq 0.05$ according to the Duncan test, and vice versa.

4. Conclusion

This study investigated the effects of lead (Pb^{2+}) contamination on the germination, growth, and physiological characteristics of *E. sativa*. The results demonstrated that soil contamination with Pb^{2+} at concentrations of 25 and 50 mg kg^{-1} significantly reduced seed germination and adversely affected several morphological and physiological parameters, including plant height, stem diameter, leaf area, root length, and stomatal characteristics (stomatal width on the adaxial surface and stomatal number on the abaxial surface). In addition, increasing Pb^{2+} concentrations significantly decreased the metal tolerance index (MTI) of the shoots, indicating reduced plant performance under lead stress. These findings confirm that lead toxicity negatively influences the growth and development of *E. sativa*, although the species exhibited a measurable degree of tolerance to Pb^{2+} exposure.

Overall, the study provides valuable insight into the impact of lead contamination on *E. sativa* and contributes to a better understanding of plant responses to heavy metal stress. Future research should investigate the physiological and molecular mechanisms underlying lead tolerance, evaluate the long-term effects of Pb^{2+} accumulation on plant productivity and food safety, and explore strategies such as soil amendments or microbial inoculation to enhance plant tolerance and reduce lead uptake under contaminated soil conditions.

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The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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No additional data are available.

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